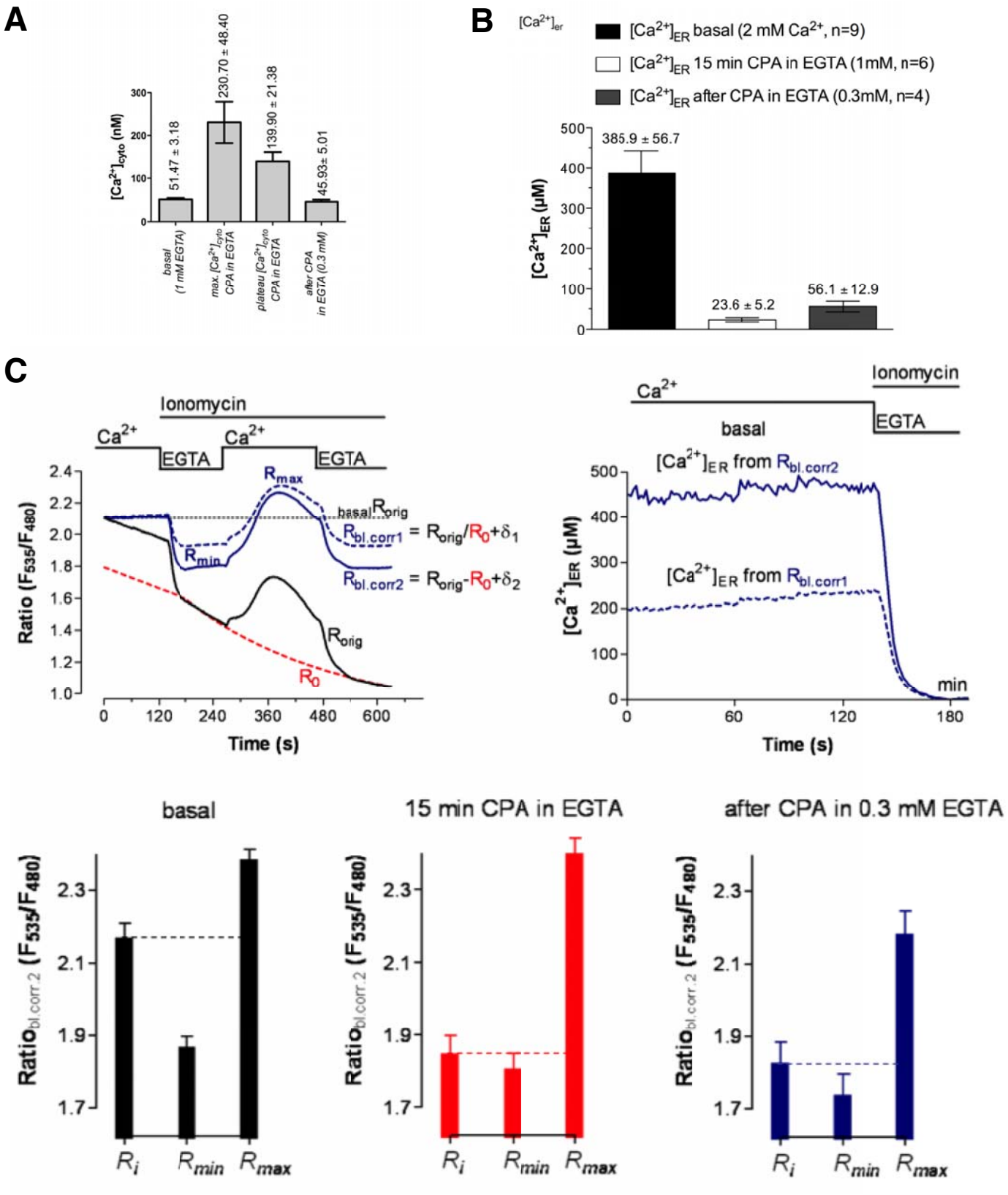
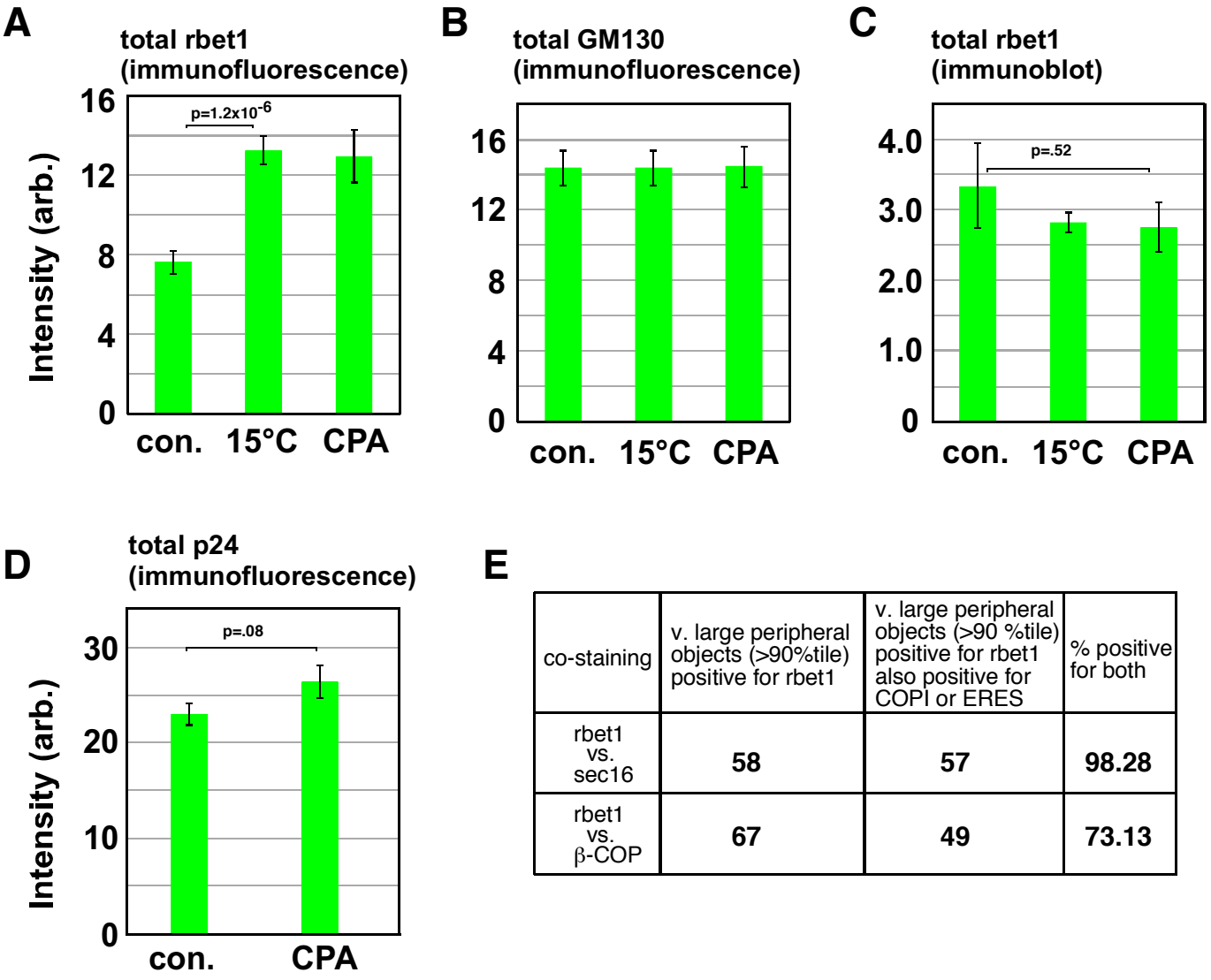


Figure S1. Calibration of free Ca^{2+} concentrations. (A) Calibrated cytosolic free Ca^{2+} concentrations from the experiment summarized in Figure 1B. (B) Calibrated luminal free Ca^{2+} concentrations from the experiment summarized in Figure 1C. (C) Detailed calibration information (see Methods).



E

co-staining	v. large peripheral objects (>90%tile) positive for rbet1	v. large peripheral objects (>90 %tile) positive for rbet1 also positive for COPI or ERES	% positive for both
rbet1 vs. sec16	**58**	**57**	**98.28**
rbet1 vs. β-COP	**67**	**49**	**73.13**

Figure S2. Total rbet1 immunofluorescence labeling increases upon luminal Ca^{2+} depletion. (A) Total immunofluorescence labeling per cell from the experiment summarized in Figure 1F. Labeling was performed with the 16G6 monoclonal antibody. (B) Total immunofluorescence labeling per cell from the experiment summarized in Figure 1F. Labeling was performed with a GM130 polyclonal antibody. (C) Relative ammounts of rbet1 present in parallel coverslips to those analyzed in (A) and (B), as assessed by immunoblotting using the 16G6 monoclonal antibody. (D) Total immunofluorescence labeling per cell from the experiment summarized in Figure 2C. Labeling was performed with a polyclonal anti-p24 antibody. (E) Table displaying correspondence of the largest rbet1-positive objects in CPA treated cells with ERES and COPI markers. Data are from detailed analysis of the largest rbet1 objects in 8 cells.

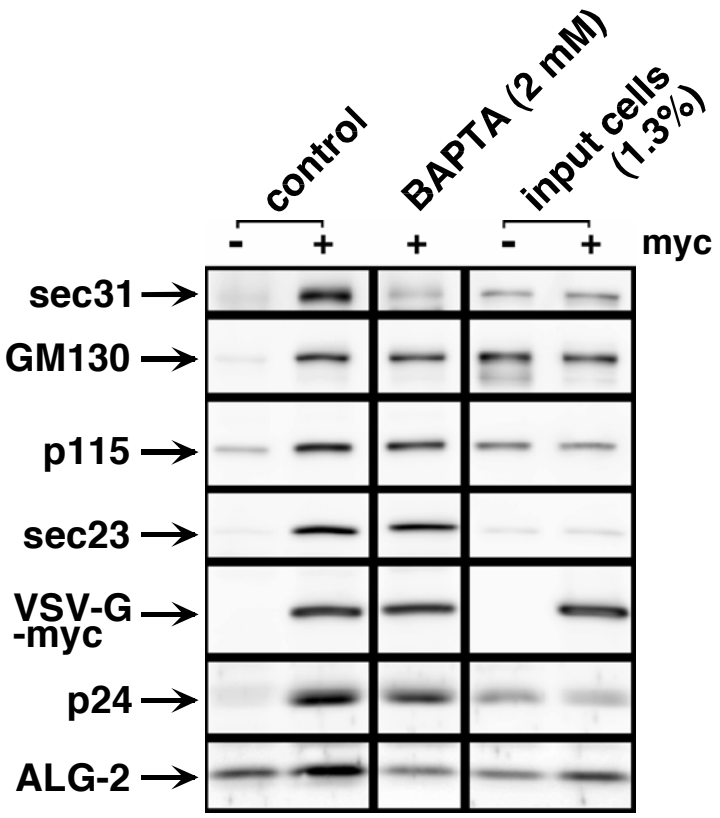


Figure S3. BAPTA specifically extracts select COPII coat-associated proteins from forming pre-Golgi intermediates. Homotypic fusion in vitro assays were conducted in the absence or presence of 2 mM BAPTA. BAPTA was added following budding so that there would be no significant effects on vesicle yield. Vesicles were derived from cells expressing VSV-G-myc (or untransfected cells as a control). Intact pre-Golgi intermediates were then immuno-isolated using anti-myc antibodies and immunoblotted for the proteins shown along the left. The recovered proteins were quantitated and normalized to the recovery of cargo VSV-G-myc in each condition (see Figure 4B).

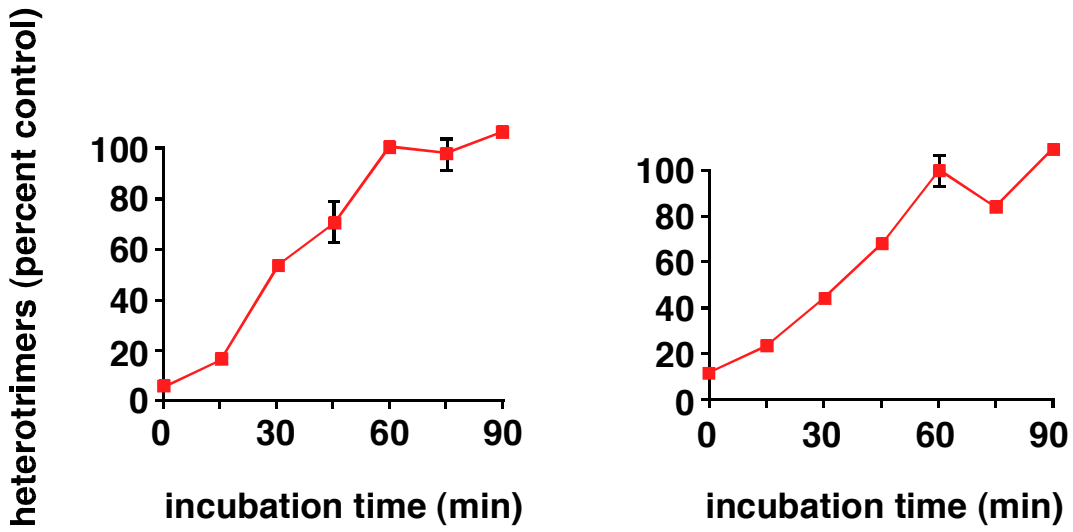


Figure S4. Representative timecourses of heterotrimerization fusion assays under the conditions used in this study. Shown are control conditions in the absence of chelators or ALG-2, for example during the 1st incubation of Figure 6B, red condition. Plotted are means of duplicate reactions, with error bars to represent standard error when exceeding symbol size. Timecourses from two distinct experiments are shown.